

## Brassicaceous Seed Meals as Soil Amendments to Suppress the Plant-parasitic Nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*<sup>1</sup>

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**Abstract:** Brassicaceous seed meals are the residual materials remaining after the extraction of oil from seeds; these seed meals contain glucosinolates that potentially degrade to nematotoxic compounds upon incorporation into soil. This study compared the nematode-suppressive ability of four seed meals obtained from *Brassica juncea* 'Pacific Gold', *B. napus* 'Dwarf Essex' and 'Sunrise', and *Sinapis alba* 'IdaGold', against mixed stages of *Pratylenchus penetrans* and *Meloidogyne incognita* second-stage juveniles (J2). The brassicaceous seed meals were applied to soil in laboratory assays at rates ranging from 0.5 to 10.0% dry w/w with a nonamended control included. Nematode mortality was assessed after 3 days of exposure and calculated as percentage reduction compared to a nonamended control. Across seed meals, *M. incognita* J2 were more sensitive to the brassicaceous seed meals compared to mixed stages of *P. penetrans*. *Brassica juncea* was the most nematode-suppressive seed meal with rates as low as 0.06% resulting in > 90% suppression of both plant-parasitic nematodes. In general *B. napus* 'Sunrise' was the least nematode-suppressive seed meal. Intermediate were the seed meals of *S. alba* and *B. napus* 'Dwarf Essex'; 90% suppression was achieved at 1.0% and 5.0% *S. alba* and 0.25% and 2.5% *B. napus* 'Dwarf Essex', for *M. incognita* and *P. penetrans*, respectively. For *B. juncea*, seed meal glucosinolate-degradation products appeared to be responsible for nematode suppression; deactivated seed meal (wetted and heated at 70 °C for 48 hr) did not result in similar *P. penetrans* suppression compared to active seed meal. *Sinapis alba* seed meal particle size also played a role in nematode suppression with ground meal resulting in 93% suppression of *P. penetrans* compared with 37 to 46% suppression by pelletized *S. alba* seed meal. This study demonstrates that all seed meals are not equally suppressive to nematodes and that care should be taken when selecting a source of brassicaceous seed meal for plant-parasitic nematode management.

**Key words:** amendment, isothiocyanate, glucosinolate, *Pratylenchus penetrans*, *Meloidogyne incognita*, brassica, seed meal.

Two global trends are occurring simultaneously that are creating opportunities in bioenergy production and plant-parasitic nematode management. There is a growing global market for biodiesel; biodiesel is a vegetable oil- or animal fat-based diesel fuel. Biodiesel output has expanded from 870 million L in 2000 to 14.7 billion L in 2008 (Earley and McKeown, 2009). In the U.S. there were 176 biodiesel plants nationwide in 2008 (Earley and McKeown, 2009). Demand for biodiesel is expected to grow. Concurrently, in the past decade several of the most effective nematicides have become unavailable (i.e., methyl bromide and fenamiphos), or their use is being restricted (i.e., 1,3-D). There is a need to provide nematode management solutions to fill these voids.

It may be possible to utilize residual materials produced by biodiesel industries for management of plant-parasitic nematodes. One such example is the seed meal produced after the extraction of oil from brassicaceous seeds. There is a large body of literature acknowledging the ability of the Brassicaceae to suppress plant-parasitic nematodes (Mojtahedi et al., 1991;

Johnson et al., 1992), soilborne pathogens (Ramirez-Villapudua and Munnecke, 1988; Subbarao et al., 1999), and weeds (Brown and Morra, 1995). Potential modes-of-action of the Brassicaceae against plant-parasitic nematodes include the production of nematotoxic glucosinolate-degradation products (i.e., isothiocyanates, thiocyanates, and nitriles) (Lazzeri et al., 1993; Zasada and Ferris, 2003), stimulation of antagonistic microbial communities in amended soil (Cohen et al., 2005), and the production of nitrogenous compounds toxic to nematodes (Cohen et al., 2005).

For plant-parasitic nematode management, members of the Brassicaceae have been applied to soil as green manures (Mojtahedi et al., 1991; Johnson et al., 1992) and as seed meal amendments (Walker, 1997; Mazzola et al., 2001). There are several advantages of using brassicaceous seed meals over growing brassicaceous green manures for plant-parasitic nematode management (Rahman and Somers, 2005): a seed meal can be easily spread and incorporated into soil, there is no risk of frost damage to the green manure plant, the brassicaceous meal will not serve as a host to plant-parasitic nematodes, and seed meal can be applied to coincide with plant-parasitic nematode population increases. Disadvantages to using brassicaceous seed meals are availability as well as cost.

The overall goal of this research program is to improve the use of brassicaceous seed meals for plant-parasitic nematode management in a diversity of cropping systems. The specific objective of this research was to compare nematode suppression by several seed meals and to identify estimates of seed meal rates needed for the suppression of *Meloidogyne incognita* and *Pratylenchus penetrans*. A secondary objective was to

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conduct research on some of the factors that may influence the efficacy of brassicaceous seed meals for plant-parasitic nematode suppression.

#### MATERIALS AND METHODS

**Nematodes:** *Meloidogyne incognita* originally isolated from a field near Salisbury, MD and cultured on greenhouse-grown pepper (*Capsicum annuum*) 'PA-136' was used in all assays. Individual egg masses were picked from roots, placed in water for 30 min, and rinsed with sterile deionized water three times. To obtain second-stage juveniles (J2), eggs were placed on a Baermann funnel to hatch; 72 hr later J2 were collected and used. Alternatively, egg masses were shaken in 0.6% sodium hypochlorite for 3 min, eggs were captured on a 25- $\mu$ m pore diam. sieve, rinsed, placed on a 25- $\mu$ m pore diam. sieve and J2 were collected in water below the mesh for 3-4 d. *Pratylenchus penetrans* originally isolated from a field near Lynden, WA and cultured on greenhouse-grown mint (*Mentha requienii*) was used in all assays. Roots from cultures were placed under intermittent mist to collect mixed stages of *P. penetrans* over a 7-d period (Ingham, 1994). Nematodes were collected each day and stored at 4 °C until used.

**Brassicaceous meals:** Four seed meals were evaluated in replicated experiments: *Sinapis alba* 'IdaGold', *Brassica napus* 'Dwarf Essex', *B. napus* 'Sunrise', and *B. juncea* 'Pacific Gold'. Seed of each variety was cold pressed removing ~90% of the oil (Peterson et al., 1983). Glucosinolate analysis was performed on each seed meal as previously described (Borek and Morra, 2005). A full elemental description of *B. juncea*, *B. napus* 'Sunrise', and *S. alba* can be found in Snyder et al. (2009).

**Comparison of seed meals for nematode suppression:** A steam pasteurized sandy soil (1:2 by volume, washed sand and Willamette loam) was used in all experiments. The soil was dried for 24 hr at 70 °C, passed through a 2-mm pore diam. sieve, and 50 g dry soil was weighed into 10 cm by 10 cm sealable bags. Each seed meal was amended to soil in bags at the following rates: 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0% dry w/w (corresponding to approximately 10, 20, 50, 100, 150, and 200 t/ha at an incorporation depth of 15 cm). A nonamended control was included in all experiments. All treatments were replicated five times and experiments conducted twice. Immediately following amendment, mixed stages of *P. penetrans* or *M. incognita* J2 were added to the soil in 7.5 ml water at an inoculation level of approximately 1,000 to 1,500 nematodes per bag. The bags containing soil, meal, and nematodes were gently massaged to ensure even distribution of nematodes and water in the bags. The contents of the bags were then poured into 50-ml polystyrene tubes, 1 ml water was added to the top of each soil/meal mixture and the tubes were incubated for 3 d, uncapped, at room temperature (approximately 25 °C). After incubation, the contents of the

tubes were placed onto tissue supported by a screen, and the screen placed onto a Baermann funnel for 3 d (Ingham, 1994). Nematodes were collected from Baermann funnels and stored at 4 °C until counted. At the time of nematode collection the amount of fungal growth on amended and unamended soils placed on Baermann funnels was assessed using the following rating system: 0 = no fungal growth; 1 = trace fungal growth; 2 = at least half of soil covered with fungal growth, and; 3 = entire surface of soil covered with fungal growth.

**Effect of *Sinapis alba* particle size on *Pratylenchus penetrans* suppression:** *Sinapis alba* was partitioned into different particle sizes by grinding, sieving, and pelletizing. The particle sizes tested were: 1) 5-mm-diam. pellets, 2) flakes > 4 mm, 3) flakes < 4 mm and > 2 mm, 4) flakes < 2 mm and > 1 mm, and 5) mustard meal ground to pass through a 841- $\mu$ m pore diam. sieve. All particle sizes were amended to 50 g of the pasteurized sandy soil at a 1% w/w rate (based upon previous experiments) and *P. penetrans* (~ 1,000 to 1,500) were added to the soil/mustard meal mix in 7.5 ml water. A nonamended control was included. All other experimental parameters were identical to those already described. Treatments were replicated five times and the experiment was conducted twice.

**Effect of deactivated *Brassica juncea* on *Pratylenchus penetrans* suppression:** *Brassica juncea* seed meal was covered with water, and then placed in a 70 °C oven for at least 48 hr to deactivate the seed meal (i.e. hydrolyze 2-propenyl glucosinolate to 2-propenyl isothiocyanate which then volatilized). The deactivated seed meal was ground to a fine powder to pass through an 841- $\mu$ m pore diam. sieve. The deactivated *B. juncea* was amended to soil at the rates 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0% dry w/w. A nonamended control was included. *Pratylenchus penetrans* (~ 1,000 to 1,500) were added to the soil/mustard meal mix in 7.5 ml water. All other experimental parameters, incubation and extraction, were identical to those already described. Treatments were replicated five times and the experiment was conducted twice.

**Data analysis:** Raw data (number of nematodes recovered from soil after treatment with brassicaceous seed meals) was  $\log_{10}(x+1)$ -transformed and percentage data (nematode reduction compared to the non-amended control) was arcsine-transformed prior to analysis when necessary to meet the assumptions of the statistical models used. Data were analyzed with the statistical package JMP (SAS Institute, Cary, NC). Differences in nematode recovery or reduction among treatments were determined by analysis of variance, and means were compared using Tukey's adjustment for multiple comparisons ( $P \leq 0.05$ ). When the assumptions of the statistical model were not met (normality and homogeneity of variance), differences in nematode recovery or reduction among treatments were

determined nonparametrically using the Kruskal-Wallis Test (ranked sums) and means were compared using Tukey's adjustment for multiple comparisons ( $P \leq 0.05$ ). Data presented are nontransformed means  $\pm$  standard errors (SE).

## RESULTS

**Brassicaceous seed meal chemistries:** The seed meals were made up of different glucosinolates, and these compounds occurred in different concentrations depending on the seed meal (Table 1). *Sinapis alba* had a very high concentration of 4-hydroxybenzyl glucosinolate, comprising 96% of its glucosinolate profile. *Brassica juncea* had the highest concentration of 2-propenyl glucosinolate, comprising 99% of its glucosinolate profile. *Brassica napus* 'Dwarf Essex' contained 3-butenyl glucosinolate and had a higher total concentration of glucosinolates than *B. napus* 'Sunrise'.

**Comparison of seed meals for nematode suppression:** Each seed meal had unique effects on the plant-parasitic nematodes (Fig. 1). When seed meal was the only variable considered in the statistical model, the order of toxicity of the seed meals regardless of concentration or plant-parasitic nematode was: *B. juncea* > (*B. napus* 'Dwarf Essex' = *S. alba*) > *B. napus* 'Sunrise' ( $P \leq 0.001$ ). Regardless of the seed meal type or concentration, *M. incognita* J2 were always more susceptible to the seed meals compared to mixed stages of *P. penetrans* ( $P \leq 0.001$ ). Data from the repeated experiments were not different for any of the seed meals ( $P > 0.05$ ), therefore the data were combined for analysis and presentation.

***Brassica napus* 'Dwarf Essex':** There was a significant interaction between seed meal rate and nematode for *B. napus* 'Dwarf Essex' ( $P \leq 0.001$ ) (Fig. 1A). At 0.5, 1.0, and 2.5%, *B. napus* 'Dwarf Essex' was more suppressive toward *M. incognita* than *P. penetrans*. At rates of 5.0% and higher there was no difference in plant-parasitic nematode suppression by *B. napus* 'Dwarf Essex'. The lowest rate (0.5%) of *B. napus* 'Dwarf Essex' when tested against *P. penetrans* resulted in nematode re-

covery that was not different from the nonamended control ( $P \leq 0.001$ ) (Fig. 1A); when suppression was calculated relative to the nonamended control this rate resulted in suppression that was different from the other rates tested. There was a dose response of *P. penetrans* to *B. napus* 'Dwarf Essex' with suppression increasing with increasing rates up to 5.0%. There was no difference in *M. incognita* suppression at rates ranging from 0.5 to 10.0% (Fig. 1A). Therefore, lower rates of *B. napus* 'Dwarf Essex' (0.05, 0.1, 0.25, and 0.5%) were tested against *M. incognita* to identify a rate which would result in < 90% suppression (data not shown). There was no difference ( $P > 0.05$ ) in nematode recovery between the lowest rate tested, 0.05%, and the nonamended control with  $51 \pm 7.8\%$  recovery. The next highest rate, 0.1% w/w, resulted in  $73 \pm 8.1\%$  suppression in *M. incognita*, and was similar to the suppression by both 0.05% and 0.25% ( $91 \pm 3.1\%$ ). The two highest rates, 0.25 and 0.5%, resulted in *M. incognita* suppression similar to each other, with  $99 \pm 0.7\%$  suppression at a rate of 0.5%.

***Brassica napus* 'Sunrise':** There was no difference in the number of *M. incognita* and *P. penetrans* recovered from *B. napus* 'Sunrise'-amended soil applied at 0.5 and 1.0% compared to the nonamended control ( $P > 0.05$ ) (Fig. 1B). At all of the rates tested, except 0.5%, there was no difference in *M. incognita* and *P. penetrans* suppression by this seed meal. Suppression of *P. penetrans* increased as the rate of *B. napus* 'Sunrise' increased up to 5.0%. There was no difference in *M. incognita* suppression at 0.5 and 1.0% *B. napus* 'Sunrise'. At rates above 5.0% there was > 95% reduction in recovery of both nematodes.

***Brassica juncea* 'Pacific Gold':** All rates of *B. juncea* resulted in almost 100% reduction in *P. penetrans* and *M. incognita* recovery compared to the nonamended control ( $P \leq 0.001$ ) (Fig. 1C). Additional experiments were conducted to identify rates that did not result in *M. incognita* or *P. penetrans* suppression (Table 2). A rate as low as 0.02% resulted in *M. incognita* suppression compared to the nonamended control; however, for *P. penetrans* there was no difference in suppression at this

TABLE 1. Glucosinolate type and concentration in the *B. juncea*, *B. napus*, and *S. alba* seed meals tested against mixed stages of *Pratylenchus penetrans* and *Meloidogyne incognita* second-stage juveniles.

Glucosinolate R-group (common name) <sup>a</sup>	<i>B. juncea</i> 'Pacific Gold'	<i>S. alba</i> 'IdaGold'	<i>B. napus</i> 'Dwarf Essex' <sup>b</sup>	<i>B. napus</i> 'Sunrise'
----- $\mu\text{mole/g}$ defatted seed meal -----				
2-propenyl (sinigrin)	152.0 $\pm$ 12.3 <sup>c</sup>	-	-	-
4-hydroxybenzyl (glucosinalbin)	-	156.8 $\pm$ 4.7	-	-
3-butenyl (gluconapin)	-	-	35.6	2.9 $\pm$ 0.1
2-hydroxy-3-butenyl (progoitrin)	-	7.1 $\pm$ 0.8	2.2	1.3 $\pm$ 0.3
4-hydroxy-3-indolylmethyl (4-hydroxyglucobrassicin)	1.2 $\pm$ 0.3	-	4.1	10.0 $\pm$ 1.0
4-methoxy-3-indolylmethyl (4-methoxyglucobrassicin)	-	-	-	0.5 $\pm$ 0.1
Total glucosinolate	153.2	163.9	41.9	14.8

<sup>a</sup> Glucosinolates were quantified following a methodology described by Borek and Morra (2005).

<sup>b</sup> Values are from one sample ( $n = 1$ ).

<sup>c</sup> Values shown are the mean of seven samples ( $n = 7$ )  $\pm$  SE.

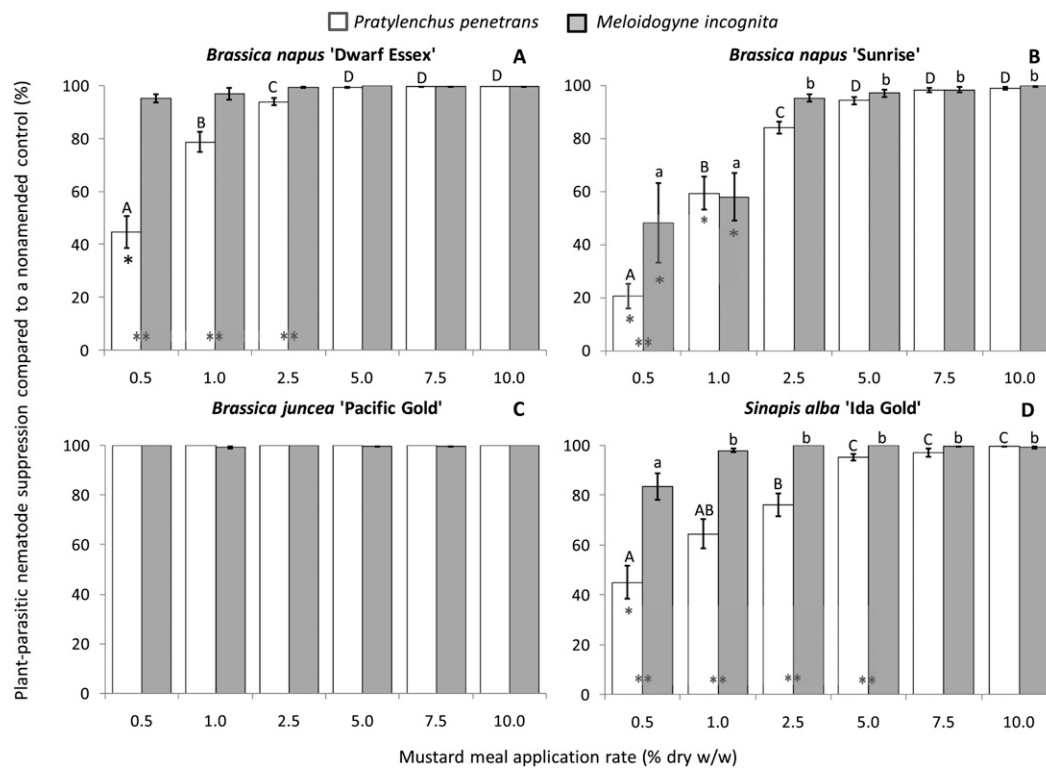


FIG. 1. Effect of brassicaceous seed meals on *Meloidogyne incognita* second-stage juveniles and mixed stages of *Pratylenchus penetrans* suppression compared to a nonamended control. Shown are the average of two trials with five replicates each ( $n = 10$ )  $\pm$  SE. \* indicates no difference ( $P \geq 0.05$ ) in nematode recovery between this treatment and the nonamended control; \*\* indicates statistically significant ( $P \leq 0.05$ ) differences in *M. incognita* and *P. penetrans* suppression at the tested rate of seed meal, and; bars within nematode species with the same letter are not statistically different ( $P \geq 0.05$ ), all according to Tukey's adjustment for multiple comparisons. Percentage data were arcsine-transformed and raw data (nematode counts) were  $\log_{10}(x+1)$ -transformed prior to analysis; nontransformed data are presented.

rate compared to the nonamended control. At 0.02% and 0.04% *B. juncea* was more suppressive to *M. incognita* than to *P. penetrans*. At the higher rates tested,  $> 0.06\%$ , there was no difference in suppression of the two nematode species by this seed meal. Exposure of nematodes to *B. juncea* at rates  $> 0.06\%$  resulted in  $\geq 90\%$  nematode suppression.

TABLE 2. Suppression of *Meloidogyne incognita* second-stage juveniles and mixed stages of *Pratylenchus penetrans* with reduced rates of *Brassica juncea* 'Pacific Gold' seed meal.<sup>a</sup>

Rate (% w/w)	% Nematode reduction	
	<i>M. incognita</i> <sup>b</sup>	<i>P. penetrans</i> <sup>b</sup>
0.02% <sup>c</sup>	90 (+3.9) A	42 (+11.5) A
0.04%	98 (+1.2) A	79 (+3.4) B
0.06	95 (+3.9) A	90 (+3.2) CB
0.08	95 (+3.4) A	96 (+1.1) DC
0.1	97 (+2.2) A	97 (+1.7) D

<sup>a</sup> Repeated experiments were similar ( $P > 0.05$ ), therefore trials were combined for analysis.

<sup>b</sup> Values shown are the mean of ten replicates ( $n = 10$ )  $\pm$  SE. Values in a column followed by different letter are significantly different ( $P < 0.001$ ) according to Tukey's adjustment for multiple comparisons. Percentage data was arcsine transformed prior to analysis; nontransformed data are presented.

<sup>c</sup> \* denotes a difference in the level of suppression between the two nematode species at this rate according to Tukey's adjustment for multiple comparisons ( $P < 0.001$ ).

*Sinapis alba* 'Ida Gold'. When *S. alba* was applied at 0.5% there was no difference in the number of *P. penetrans* recovered between this rate and the non-amended control ( $P \leq 0.001$ ) (Table 1D). There was a significant interaction between concentration and nematode for *S. alba* ( $P \leq 0.001$ ). At rates up to 5.0% *M. incognita* suppression was greater than suppression of *P. penetrans*. As the rate of *S. alba* increased up to 5.0%, so did suppression of *P. penetrans*. A similar trend was observed for *M. incognita*, but only up to 1.0%. *Sinapis alba* seed meal applied at  $> 5.0\%$  resulted in  $> 95\%$  reduction regardless of nematode.

*Fungal growth on soil amended with brassicaceous seed meals:* The seed meals differed in their ability to promote/support fungal growth in amended soils. *Brassica juncea* supported almost no fungal growth regardless of rate compared to the other seed meals (data not shown). Using a rating scale of 0 to 3, across all rates (0.5% to 10%), *B. napus* 'Dwarf Essex' supported more fungal growth with an average score of  $1.7 \pm 0.1$  than *B. napus* 'Sunrise' and *S. alba*, with average scores of  $1.1 \pm 0.1$  and  $1.2 \pm 0.1$ , respectively ( $P \leq 0.001$ ).

*Effect of Sinapis alba 'Ida Gold' particle size on Pratylenchus penetrans suppression:* Trials were significantly different ( $P = 0.02$ ), therefore the data are presented separately. In both trials, only the pellet-sized particles



(the largest one tested) did not reduce recovery of *P. penetrans* compared to the nonamended control (Table 3). In Trial 1 ground *S. alba* seed meal applied to soil resulted in the greatest reduction in recovery compared to the other particle sizes. However, in Trial 2, there was no difference in nematode reduction among the ground, small- and medium-sized particles ( $P \leq 0.001$ ). Consistent between the two trials was the fact that the larger particles sizes, large and pellet, resulted in the same level of nematode reduction and were different from the ground material.

**Effect of deactivated *Brassica juncea* 'Pacific Gold' on *Pratylenchus penetrans* suppression:** There were only trace amounts of 2-propenyl glucosinolate remaining in the *B. juncea* seed meal that was deactivated by hydrolysis and elevated temperature. When the deactivated *B. juncea* seed meal was applied to soil the results were very different from those obtained by active *B. juncea* seed meal (Figs. 1C and 2). *Pratylenchus penetrans* suppression by deactivated seed meal applied at 0.5% was not different from the nonamended control ( $P > 0.05$ ) (Fig. 2). This was in sharp contrast to the results from the activated *B. juncea* seed meal where all rates resulted in almost a 100% reduction in *P. penetrans* survival. Between the rates 0.5% and 5.0% deactivated *B. juncea* seed meal resulted in a significant ( $P \leq 0.001$ ) increase in the *P. penetrans* suppression as the rate increased. It is interesting to note that fungal growth was promoted/supported in deactivated *B. juncea* seed meal (data not shown). This is in contrast to the lack of fungal growth in active *B. juncea* meal (see above).

## DISCUSSION

This research demonstrates that knowledge of the sensitivity of the target plant-parasitic nematode to

TABLE 3. The effect of *Sinapis alba* 'IdaGold' seed meal particle size on mixed stages of *Pratylenchus penetrans* compared to a non-amended control.<sup>a</sup>

<i>S. alba</i> particle size <sup>b</sup>	% <i>P. penetrans</i> reduction	
	Trial 1 <sup>c</sup>	Trial 2 <sup>c</sup>
Ground	93 ( $\pm 1.4$ ) A	93 ( $\pm 1.4$ ) A
Small	66 ( $\pm 6.9$ ) B	81 ( $\pm 5.8$ ) AB
Medium	75 ( $\pm 29$ ) B	80 ( $\pm 4.2$ ) AB
Large	57 ( $\pm 11.0$ ) BC	67 ( $\pm 4.0$ ) BC
Pellet	37 ( $\pm 3.9$ ) C* <sup>d</sup>	46 ( $\pm 3.7$ ) C*

<sup>a</sup> Nematodes were added to 50 g dry soil amended with 1.0% w/w *S. alba*.

<sup>b</sup> Particle size designations are: ground = ground to pass through a 20 mm-mesh (841  $\mu$ m) sieve in a Wiley Mill; small = flakes < 2 mm and > 1 mm; medium = flakes < 4 mm and > 2 mm; large = flakes > 4 mm; and, pellet = single piece of pellet with a diameter of approximately 5 mm.

<sup>c</sup> Trials were significantly different at  $P = 0.02$ . Values shown are the mean of four replicates ( $n = 4$ )  $\pm$  SE. Values in a column followed by different letter are significantly different ( $P < 0.001$ ) according to Tukey's adjustment for multiple comparisons. Percentage data were arcsine transformed prior to analysis; nontransformed data are presented.

<sup>d</sup>\* denotes that this treatment was not statistically different ( $P \geq 0.05$ ) from the nonamended control according to Tukey's adjustment for multiple comparisons. Data were  $\log_{10}(x+1)$  transformed prior to analysis; nontransformed data are presented.

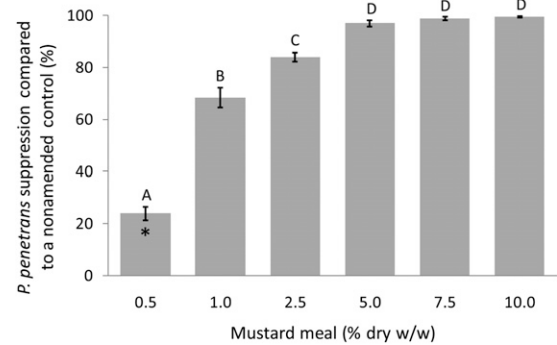


FIG. 2. Effect of deactivated *Brassica juncea* seed meal on mixed stages of *Pratylenchus penetrans* compared to a nonamended control. *Brassica juncea* seed meal was wetted and heated at 70°C for 48 hr and ground to pass through a 20-mm sieve. Shown are the average of two trials with five replicates each ( $n = 10$ )  $\pm$  SE. \* indicates no statistically significant ( $P \geq 0.05$ ) difference in nematode recovery between this treatment and the nonamended control and bars with the same letter are not statistically different ( $P \geq 0.05$ ), both according to Tukey's adjustment for multiple comparisons. Percentage data were arcsine-transformed and raw data (nematode counts) were  $\log_{10}(x+1)$ -transformed prior to analysis; nontransformed data are presented.

a seed meal may help improve efficacy as well as the economics of utilizing brassicaceous seed meals as a nematode management strategy. This is the first time that several seed meals, applied at a range of rates, have been tested side-by-side against two economically important plant-parasitic nematodes. There was a tremendous amount of variability in the efficacy of the seed meals against *M. incognita* and *P. penetrans*. *Meloidogyne incognita* was more susceptible to the brassicaceous seed meals than *P. penetrans*. This contradicts a previous findings which evaluated *B. juncea* 'Forge' against several plant-parasitic nematodes and found *P. penetrans* to be more sensitive (393  $\mu$ g/ml *B. juncea* seed meal) than *M. incognita* (474  $\mu$ g/ml *B. juncea* seed meal) (Yu et al., 2007). However, care must be taken in comparing the present soil-based study with the aqueous-based assay used by Yu et al. (2007). Plant-parasitic nematodes have also been shown to differ in their sensitivity to isothiocyanates, one of the degradation products of enzymatic glucosinolate hydrolysis (Buskov et al., 2002; Zasada and Ferris, 2003).

*Brassica juncea* was the most active seed meal tested. In fact, the rate of *B. juncea* necessary to result in 90% *M. incognita* and *P. penetrans* suppression was approximately 100-fold less than that of the other tested seed meals. *Brassica juncea* seed meal was superior to *B. napus* and *S. alba* seed meals in suppressing *P. penetrans* in apple (Mazzola et al., 2007, 2009). The lower rates (0.02% to 0.1% w/w) of *B. juncea* tested against *P. penetrans* and *M. incognita* in this study were similar to a rate (0.025 % w/w) needed to suppress *P. penetrans* on sweet corn (Yu et al., 2007). In the short-term assay employed in this study, the toxicity of the 2-propenyl glucosinolate-degradation product 2-propenyl isothiocyanate can probably be implicated. 2-Propenyl isothiocyanate is

known to be highly toxic to nematodes (Lazzeri et al., 1993; Donkin et al., 1995; Buskov, 2002; Zasada and Ferris, 2003). In this study at rates ranging from 0.5% to 10% the nematodes were potentially exposed to 7.2 to 144.0  $\mu\text{mol}$  of 2-propenyl isothiocyanate, respectively, if a 19% conversion from glucosinolate to isothiocyanate is assumed (Brown et al., 1991). 2-Propenyl isothiocyanate being the primary mode of nematode suppression by *B. juncea* is further supported by the fact that lower rates (0.5, 1.0, and 2.5%) of deactivated *B. juncea* meal not containing 2-propenyl isothiocyanate did not result in 100% nematode suppression.

Two *B. napus* seed meals, obtained from different varieties, were screened against *P. penetrans* and *M. incognita*. The ability of these seed meals to suppress the plant-parasitic nematodes, as well as their chemistries, was different. *Brassica napus* 'Dwarf Essex' resulted in greater nematode suppression than 'Sunrise', and this difference may have been associated with differences in chemistry. *Brassica napus* 'Dwarf Essex' had a higher total concentration of glucosinolates as well as a higher concentration of 3-butenyl glucosinolate compared to *B. napus* 'Sunrise'. Lazzeri et al. (1993) evaluated a mix of glucosinolates isolated from *B. napus* 'Jet Neuf' and their enzymatic degradation products against *Heterodera schachtii* in aqueous assays. While nematode mortality by the enzymatic degradation products was dependent upon concentration and exposure time, the glucosinolates from *B. napus* 'Jet Neuf' resulted in 98% mortality in a 5% solution after 48 hr. Lazzeri et al. (1993) also tested 3-butenyl glucosinolate in the presences of myrosinase against *H. schachtii* and obtained 93% mortality after 24 hr exposure to a 0.5% w/v concentration. *Brassica napus* 'Sunrise' contained 10.5  $\mu\text{mol/g}$  of indole glucosinolates that produce ionic thiocyanate, a compound that is toxic to microorganisms (Smith et al., 1945). The toxicity of ionic thiocyanate to plant-parasitic nematodes is unknown.

*Sinapis alba*'s glucosinolate profile was dominated by 4-hydroxybenzyl glucosinolate. Lazzeri et al. (1993) noted the meager nematicidal effect shown by the enzymatic-degradation products of 4-hydroxybenzyl glucosinolate against *H. schachtii*. While they attributed this lack of activity to the limited toxicity of 4-hydroxybenzyl isothiocyanate, in fact inactivity was more likely to be associated with ionic thiocyanate. 4-Hydroxybenzyl glucosinolate degrades to 4-hydroxybenzyl isothiocyanate, which is unstable in aqueous media and rapidly hydrolyzes to ionic thiocyanate at pH values expected in most soils (Borek and Morra, 2005).

While the short duration of our assays may have favored a chemical mechanism of nematode suppression by the brassicaceous seed meals, the activity of seed meals against plant-parasitic nematodes cannot be attributed solely to a single mechanism. This is evident by the fact that at rates  $\geq 5.0\%$  of any of the seed meals, active or deactivated,  $> 90\%$  nematode suppression was

achieved. Also, when deactivated *B. juncea* meal was amended to soil at 0.5% and 2.5% *P. penetrans* was suppressed by 24 and 84%, respectively. Glucosinolate hydrolysis products did not appear to have a significant role in the suppression of *Rhizoctonia* spp. or *Pratylenchus* spp. obtained by *B. napus* seed meal amendment (0.25% w/w) (Mazzola et al., 2001; Cohen et al., 2005). However, *B. napus* seed meal did alter communities of saprophytic soil micro-organisms (Cohen et al., 2005). In another study (Hoagland et al., 2008), *B. napus* seed meal increased resident populations of *Pythium* spp. while *B. juncea* did not. Fungal growth was stimulated by *B. napus* 'Dwarf Essex' and 'Sunrise' as well as *S. alba* in our assays.

Another potential mechanism of nematode suppression by brassicaceous seed meals is through nitrogenous degradation products. Seed meals have a nitrogen content of approximately 6% (Cohen et al., 2005; Snyder et al., 2009). When soybean (*Glycine max*) seed meal was applied at a similar nitrogen content to that of *B. napus* seed meal, soybean seed meal was as effective as *B. napus* seed meal in reducing numbers of *Pratylenchus* spp. recovered from the roots of apple seedlings (Cohen et al., 2005). Nitrogenous amendments have received considerable attention because of their nematicidal properties (Rodríguez-Kábana, 1986). A negative linear relationship was found between the nitrogen content in amendment materials applied to soil and *Meloidogyne* spp. infestation of plants (Rodríguez-Kábana et al., 1987). Nitrogenous products (i.e., ammonia, nitrous acid) were not measured in these experiments. Future research should strive to understand the role that nitrogen plays in the ability of brassicaceous seed meals to suppress nematodes.

We must endeavor to identify rates and develop application technologies which make nematode suppression by the brassicaceous seed meals more consistent, reliable, and economical. One factor that we considered in this research was the particle size of *S. alba* seed meal. Suppression of *P. penetrans* was greatly influenced by *S. alba* meal particle size, with ground material being more effective than larger sized particles. Intuitively this makes sense; when smaller particles are used a more even distribution of product can be achieved in soil, and the chance that a nematode will encounter the nematotoxic glucosinolate-degradation products increases. It is likely that large particle sizes create pockets of toxicity to which all nematodes are not exposed. While this may not be an issue with a 2-propenyl glucosinolate-containing seed meal (i.e., *B. juncea* or *B. carinata*), which degrades to the relatively water insoluble and volatile compound 2-propenyl isothiocyanate, particle size will be an issue for the distribution of nonvolatile and water-soluble compounds like ionic thiocyanate produced by *S. alba*. While particle size (powder and flake) of *B. napus* meal did not alter suppression of *R. solani*, it did impact the number and activity of *Pythium* spp. with the flake form resulting

in higher numbers than the powdered form (Cohen and Mazzola, 2005).

In the future, as the biodiesel industry expands, there is likely to be more brassicaceous seed meal available. Our study demonstrates that not all seed meals equally suppress nematodes, and that care should be taken when selecting a source of brassicaceous seed meal for plant-parasitic nematode management. *Pratylenchus penetrans* may be much more difficult to suppress with lower rates ( $\leq 2.5\%$ ) of *B. napus* 'Dwarf Essex' and 'Sunrise' as well as with *S. alba*. For *M. incognita*, rates  $\leq 0.5\%$  of *B. napus* 'Dwarf Essex' and *B. juncea*, and  $1.0\%$  of *S. alba* will likely be suppressive. However, efficacy will depend upon the soil environment into which the seed meal is incorporated (Gimsing and Kirkegaard, 2009) as well as the source and chemical composition of the seed meal. For brassicaceous seed meals to become a consistent and reliable nematode management strategy further consideration should be made towards application technology (i.e., particle size, timing of application, irrigation) and combining this management practice with other plant-parasitic nematode practices (i.e. reduced rates of metam sodium, cover crops).

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